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THE BACTERIOLOGICAL AND CHEMICAL BEHAVIOR OF SILVER IN LOW CONCENTRATIONS

Cecil W. Chambers, et al

Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio

1960

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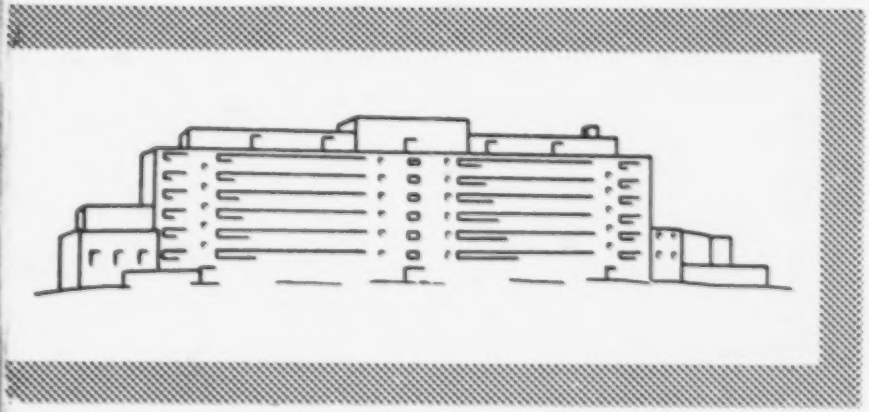

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U. S. DEPARTMENT OF HEALTH,
EDUCATION, AND WELFARE
Public Health Service

THE BACTERIOLOGICAL AND CHEMICAL BEHAVIOR OF SILVER IN LOW CONCENTRATIONS

Cecil W. Chambers,
Microbiology

Charles M. Proctor,
Chemistry and Physics

Water Supply and Water Pollution Research

U. S DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Bureau of State Services
Division of Water Supply and Pollution Control

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NOTES

The present address of co-author Charles M. Proctor is Applied Physics Section, Aero-Space Division, Boeing Airplane Company, Seattle 24, Washington.

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THE BACTERIOLOGICAL AND CHEMICAL BEHAVIOR OF SILVER IN LOW CONCENTRATIONS

Introduction

The effect of oligodynamic silver on microbiological forms was first recognized by Raulin (1869) who reported that it was toxic to some algae. Much consideration has been devoted to whether silver, when added to water by mechanisms such as filter beds, electrolytic devices, or as colloidal suspensions, has greater germicidal efficiency than the same amount of silver added as $AgNO_3$. Most of the work reported in the literature has been inadequate because of failure to recognize the importance of one or more of the following basic considerations: (1) Accurate analytical methods, (2) recognition of and compensation for adsorption of silver by glassware, (3) suitable procedures for removing toxic residuals from glassware, (4) adequate neutralizers to arrest germicidal or bacteriostatic action in bacteriological tests, and (5) suitable controls. In the present investigation, improved bacteriological and chemical methods, including radioisotope Ag^{110m}, have been used to study the factors affecting the practical application of silver germicides in water treatment. Concentrations of silver are expressed either in terms of dissolved or suspended agent determined by analysis at the time of the test or in terms of silver quantitatively added.

When the bacterial kills obtained in considerable numbers of identical daily experiments are compared, inconsistent results occur more frequently with silver than with other germicides. Replicate tests in a single experiment usually have shown good agreement. When factors such as 2 silver sources or 2 pH ranges are compared in parallel replicate tests in a give experment, clear cut trends are evident. Absolute values, such as the time required to secure a specified percent kill at a fixed germicide concentration vary more in day to day experiments with silver than with nonmetallic bactericides. This pattern is illustrated by data from 2 experiments presented in Figure 1. The results in both experiments, in which the same amount of the different types of silver was added to replicate flasks show no significant difference in the rate of kill. However, the percent of bacteria killed in any specified time, is considerably different in the two experiments.

Mineral content was not a factor because distilled water was used in the tests. Variations in silver adsorbed on glass surfaces between replicate tests in a given experiment were not a significant factor because replicate results showed good agreement. While some silver was adsorbed by the glass, the amount of silver lost in the various experiments should be essentially constant.

Germicidal efficiency of different types of silver

There has been considerable discussion concerning the germicidal efficiency of equivalent amounts of silver obtained from silver sources having grossly different physical properties. Using the rhodanine test to determine test concentrations of silver, the efficiency of 40 ppb of silver as $AgNO_3$ was compared with 40 ppb of silver in the effluent water from a silver yielding filter device. Under these conditions, as determined in preliminary analytical studies with known amounts of silver, the values for silver concentrations obtained in individual tests with the rhodanine method did not vary more than $\pm$ 7.0 percent from the calculated values.

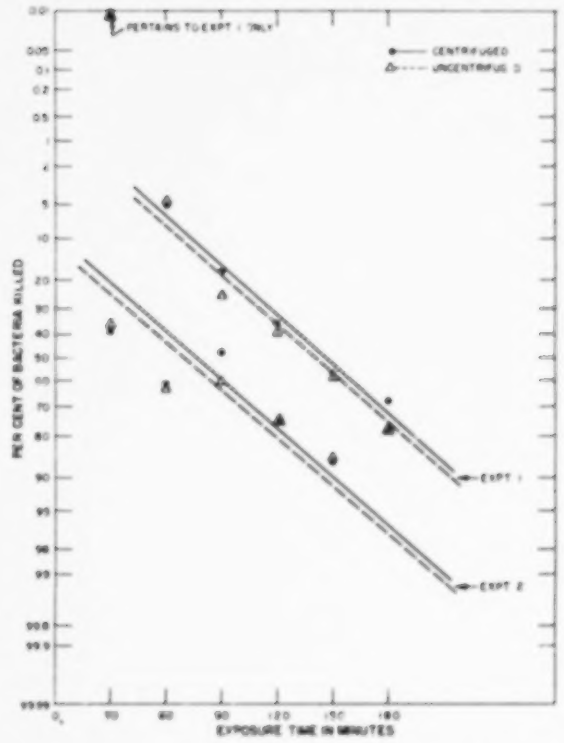

Figure 1. EFFECT OF CENTRIFUGING ON A COLLOIDAL SILVER PREPARATION
(Equal volumes of centrifuged and uncentrifuged material used in all tests)

Sufficient replication of tests was included to further reduce this experimental error.

Pyrex flasks, previously equilibrated with 40 ppb of the forms of silver considered, were used in these tests. In some of the later tests solutions containing 200 ppb of silver were used to equilibrate the glassware. The equilibration solutions were changed once or twice daily for several days. Even after such treatment some silver was adsorbed from the test solutions. Attempts to equilibrate Vycor were not significantly more successful than those with Pyrex. Equilibration of replicate flasks resulted in about the same degree of adsorption with both types of silver. Approximately 12 percent of the silver in the test material was lost to the glass during the bactericidal tests, but substantially parallel conditions of examination were maintained. Data obtained in these experiments are presented in Table 1. The similarity of results obtained with both types of silver, one of which ($AgNO_3$) was 99.9+ percent ionic (Hunt 1911) supports the concept that the germicidal factor was the silver ion.

Portions of a highly concentrated, opaque, dark colored, colloidal silver preparation were centrifuged at an average rate of 100,000 x gravity for 3-1/2 hours. A crystal clear supernatant resulted. Samples of the supernatant and of the uncentrifuged colloidal silver material were examined in parallel bactericidal tests in distilled water to which identical volumes of each had been added. Data obtained in these studies are presented in Figure 1. Because of the nature of the colloidal material chemical analyses of the diluted test menstrua were not possible.

TABLE 1. COMPARISON OF SILVER NITRATE WITH SILVER FROM A FILTER DEVICE
40 ppb Ag[1] IN DISTILLED WATER AT pH 6.1

Average[2] percent of bacteria surviving for time indicated											
30 Min.		60 Min.		90 Min.		120 Min.		150 Min.		180 Min.	
$AgNO_3$	F[3]	$AgNO_3$	F	$AgNO_3$	F	$AgNO_3$	F	$AgNO_3$	F	$AgNO_3$	F
94.4	93.1	78.9	77.7	63.3	63.5	52.4	47.3	36.0	30.3	12.7	18.4

(1) By the rhodanine test at start of experiment.
(2) Average of 3 experiments (3 replicate flasks per experiment with each type of silver).
(3) F is silver from filter device.

The test water receiving the uncentrifuged colloidal material contained relatively enormous amounts of total silver when compared with that containing the same volume of supernatant. However, the bactericidal effect with both materials was practically the same. This suggests that both the supernatant and the uncentrifuged stock material were saturated solutions of silver ions with the dispersed particulate silver serving only to provide a source of ions. Because there was no marked acceleration in kill with the uncentrifuged material in bactericidal tests as observed during 2-1/2 hours, the rate of ion release by the dispersed material to the surrounding water must have been slow. The apparent minor increase in kill with the uncentrifuged material is attributed to a slight release of ions by the diluted uncentrifuged colloidal material.

Effect of environmental factors on the germicidal efficiency of silver nitrate

CELL CONCENTRATION

Data presented in Table 2 show that at a silver concentration of less than 35 ppb, a 1000 - fold increase in the number of test bacteria did not significantly influence the rate of kill.

PHOSPHATE BUFFER

The effect of buffer salts in germicidal tests could be important. A scheme was therefore designed to determine whether the phosphate present in buffers would influence the results obtained. The tests were made at pH 6.2, the pH of the distilled water. Aliquots of distilled water were buffered with quantities of KH_2PO_4 and Na_2HPO_4 sufficient to yield a $\frac{1}{1500}$ M concentration of phosphate having a pH of 6.2. Control of the silver concentration was maintained by radio-assay methods in parallel bactericidal tests with the two waters. The data presented in Table 3 indicate that $\frac{1}{1500}$ M phosphate interferes to a moderate degree with the germicidal action of silver, possibly due to complexing of the silver.

HYDROGEN ION CONCENTRATION

The effect of pH on the germicidal activity of silver was evaluated in parallel tests at pH 6.2 and 7.5. In these experiments the phosphate concentration was maintained at a constant level by varying the ratio of equimolar solutions of disodium and potassium dihydrogen phosphate. The working solution was $\frac{1}{1500}$ M. The results presented in Table 4 show an increased kill rate at the higher pH. These findings confirm those of Chambers, Chambers,

TABLE 2.(1) RELATIONSHIP OF BACTERIAL DENSITY TO GERMICIDAL EFFICIENCY(2) AT pH 7.0

Germicide	Bacteria per ml.	Percent surviving for:		
		2 Hrs.	4 Hrs.	6 Hrs.
Control	1,790	83.8	82.7	87.2
	1,800,000	99.1	92.9	94.0
$AgNO_3$	1,680	44.6	33.0	20.2
	1,700,000	44.8	28.5	28.7

(1) Representative experiment.
(2) Ag concentration less than 35 ppb.

TABLE 3. GERMICIDAL ACTION OF SILVER NITRATE IN PHOSPHATE BUFFER AND DISTILLED WATER AT pH 6.2

Test water	ppb Ag^{110m} (1)	Average(2) percent of bacteria surviving for:			
		1 Hr.	2 Hrs.	3 Hrs.	4 Hrs.
Phos.	89	40.1	4.5	0.30	0.028
Dist.	90	22.0	1.22	0.08	0.005

(1) Silver present at the start of an experiment. With distilled water an average of 13.1 percent of the Ag present was lost to the glass; the corresponding loss with phosphate water was 3.3 percent.
(2) Av. of 2 experiments.

and Kabler (1953), and Wuhrmann and Zobrist (1958).

LIGHT

Because silver in some forms is very sensitive to the action of light it was considered possible that light might affect the bactericidal efficiency of silver ions. The data presented in Table 5 were obtained in parallel tests with $AgNO_3$ in which half of the replicate flasks were kept in the dark and the other half exposed to room light, a mixture of fluorescent tube light and daylight (northern exposure to large windows in September). The effect of light was not important under the conditions of these tests.

GERMICIDAL EFFECT OF SILVER ADSORBED ON PYREX GLASS

In parallel tests, in each of several daily experiments, a known concentration of $AgNO_3$ was placed in half of the replicate flasks 18-1/2 hours before the bacterial suspension was added and in the remaining flasks immediately before the organisms were added. The data obtained in these experiments are presented in Table 6.

Much of the germicidal capacity was lost during the 18-1/2 hour interval. Ag^{110m} procedures were not available at the time the work reported in Table 6 was done but later work with Ag^{110m} showed that 15 to 45 percent of the Ag^{110m} was adsorbed on flasks (p. 6) in this length of time. These losses could explain the decrease in germicidal activity reported in Table 6.

Pyrex glass tubes adsorbed silver from solution, only part of which was removed by dichromate cleaning solution treatment followed by a flow-through rinse. Some of this silver was desorbed and exerted a germicidal action when the glass was reused. More silver was adsorbed on surfaces in contact with strong solutions of silver than from relatively weaker solutions as will be shown later. Table 7 shows the micrograms of silver adsorbed per tube surface and the subsequent germicidal effect, on contact with bacterial suspensions, of silver released by that portion of the adsorbed silver which remained after dichromate cleaning.

TABLE 4. EFFECT OF pH[1] ON GERMICIDAL EFFICIENCY OF $AgNO_3$

ppb[2] Ag	pH	Percent of bacteria surviving for:							
		15 Min.	30 Min.	45 Min.	60 Min.	75 Min.	90 Min.	105 Min.	120 Min.
38	6.2	86.8	65.8	58.4	35.9	49.7	31.3	17.3	14.5
38	7.5	75.8	34.7	10.4	1.93	0.84	0.03	0.02	0

(1) PO_4 concentration constant at $\frac{1}{1500}$ M.

(2) 40 ppb added - value shown is amount determined by rhodanine test at end of experiment.

TABLE 5.[1] EFFECT OF LIGHT ON GERMICIDAL EFFICIENCY OF $AgNO_3$ IN DISTILLED WATER AT pH 6.1

Light status	Ag[2]	Percent of bacteria surviving for:							
		15 Min.	30 Min.	1 Hr.	2 Hrs.	3 Hrs.	4 Hrs.	5 Hrs.	6 Hrs.
Light	35	84.7	79.4	62.9	11.2	3.72	1.42	0.64	0.53
Dark	35	100.0	74.1	57.2	6.27	7.28	2.41	1.25	0.03

(1) Representative experiment.

(2) Amount of Ag initially added. No final analysis.

TABLE 6.(1) EFFECT OF CONTACT WITH GLASS ON GERMICIDAL EFFICIENCY OF $AgNO_3$ IN DISTILLED WATER AT pH 6.2

ppb Ag(2)	Contact time(3)	Percent of bacteria surviving for:							
		15 Min.	30 Min.	1 Hr.	2 Hrs.	3 Hrs.	4 Hrs.	5 Hrs.	6 Hrs.
35	1 Hr.	38.1	37.1	29.0	16.7	8.90	3.43	0.81	0.84
35	18-1/2 Hrs.	86.2	71.4	60.5	36.7	27.6	16.0	11.8	5.96
70	1 Hr.	61.4	38.1	31.9	8.0	1.10	-0.01	0	0
70	18-1/2 Hrs.	71.4	61.9	41.9	15.0	6.57	0.26	0.07	0

(1) Representative experiment.
(2) Amount of Ag initially added.
(3) Elapsed time between adding test solution to flask and adding test suspension.

TABLE 7. GERMICIDAL EFFECT OF DESORBED Ag^{110m}

Tube No.(1)	ppm Ag^{110m}(2)	Micrograms Ag^{110m}(3) adsorbed	Percent of bacteria surviving for:		
			2 Hrs.	4 Hrs.	6 Hrs.
Control(4)	10,000	3.00	0	0	0
Av. 131 & 141	1,000	2.92	9.47	0	0
142	320	2.35	8.33	1.66	0
143	100	3.38	6.10	0.76	0
Av. 135 & 145	10	1.86	0.35	0	0
Av. 137 & 147	1	1.92	3.20	0.80	0
Av. 328 & 329	0.2	0.27	96.1	86.1	76.1
Colloidal(5)	Undiluted(6)	...	18.0	3.32	1.72

(1) Full of $Ag^{110m}NO_3$ solution during contamination period.
(2) In toxifying solution.
(3) The amount of adsorbed silver remaining after dichromate cleaning is not reported because tubes could not be counted subsequent to dichromate treatment due to danger of bacterial contamination by probe. However, tubes treated in a parallel fashion were counted and showed that dichromate cleaning removed 35-60 percent of the residual silver.
(4) Flow-through rinse only, no subsequent dichromate cleaning.
(5) Colloidal Ag^{110m} was not available.
(6) Undiluted gross material in commercial product was used - actual Ag content not known.

Neutralizer studies

In a neutralizer test, aliquots of a neutralizer-disinfectant mixture containing 2 ppm of silver as $AgNO_3$ in 730 ppm of $Na_2S_2O_3 \cdot 5H_2O$ were inoculated at intervals of 0, 2, 4, and 6 hours after adding silver and culture suspension. At each time interval 2 portions were cultured in nutrient agar pour plates, 2 parallel inoculations also being made in nutrient agar containing 500 ppm of sodium thioglycollate. The results of these experiments are presented in Table 8. These data show that the sodium thiosulfate neutralizer protected all of the bacteria from the bactericidal action of the silver for 6 hours, but failed to neutralize the bacteriostatic effect which had prevented growth in plain agar cultures.

A mixture of sodium thiosulfate and sodium thioglycollate was found to be a good neutralizer.

TABLE 8. EFFICIENCY OF $AgNO_3$ NEUTRALIZATION

Ag(1) ppm	Composition of neutralizer	Percent of bacteria surviving for:							
		0 Hrs.		2 Hrs.		4 Hrs.		6 Hrs.	
		N(2)	T(3)	N(2)	T(3)	N(2)	T(3)	N(2)	T(3)
10.0		75.8	100.0	17.9	55.9	13.9	43.1	1.29	24.0
5.0	1000 ppm Na_2SO_3	91.9	100.0	18.1	40.4	17.9	30.6	9.90	26.5
1.0		100.0	100.0	70.0	78.9	52.2	76.4	40.4	69.4
10.0	A mixture of 500 ppm sodium			100.0	98.8	92.5	94.7	95.0	87.1
5.0	thioglycollate and 730 ppm			99.4	100.0	99.0	100.0	100.0	91.5
1.0	$Na_2S_2O_3 \cdot 5H_2O$			100.0	92.3	93.8	100.0	97.1	90.4
10.0	A mixture of 2000 ppm of glyc-	81.8	100.0	4.53	24.7	0	20.6	0	14.8
5.0	erol and 1000 ppm Na_2SO_3			12.3	43.0	3.60	27.6	4.96	21.8
1.0				60.7	87.2	42.0	65.2	38.8	59.0
20.0		0	98.2	0	84.0	0	98.0	0	100.0
10.0	730 ppm $Na_2S_2O_3 \cdot 5H_2O$	25.0	92.4	52.2	100.0	60.3	97.3	5.88	100.0
5.0		93.6	72.0	97.3	86.9	100.0	98.7	49.9	100.0

(1) Concentration of Ag in 1 ml. aliquot added to 9 ml. of neutralizer.
(2) Plain nutrient agar.
(3) Nutrient agar containing 500 ppm sodium thioglycollate.

Data indicating this, along with those obtained with less effective neutralizers are also presented in Table 8. Simultaneous germicide tests with a high concentration of a colloidal silver preparation indicated that the sodium thiosulfate-thioglycollate mixture was also effective in neutralizing this material. Results obtained with a mixture of sodium thiosulfate and sodium thioglycollate were more consistent than those with sodium thioglycollate alone. The apparent superiority of the mixture may reflect the statistical limitations of the data obtained in a lesser number of tests with the thioglycollate.

Absorption of silver on surfaces

The tendency of silver to be adsorbed on many types of materials causes 3 particularly troublesome experimental problems: (1) adsorption of silver from solution during bactericidal tests results in a progressive reduction in the silver concentration, (2) the adsorbed silver is not effectively removed by conventional laboratory cleansing procedures, (3) part of the adsorbed silver is subsequently released when such equipment is reused later. Because silver is so highly bacteriostatic the disposition or segregation of silver contaminated equipment in the laboratory, and particularly in laboratories having centralized cleanup and preparation facilities, is a serious problem.

GENERAL CONSIDERATIONS

The adsorption of silver varies with the strength and composition of the solution and the kind of material used in the container, and is also affected by the previous treatment of the container. Hydrogen ions and various silver complexing agents such as halides, phosphates, and ammonia, reduce the amount of adsorption. In tests using several dozen 250 ml Erlenmeyer Pyrex flasks, adsorption of silver from a neutral solution containing about 100 ppb of Ag^{110m} ranged from 15-45 percent. This adsorption was usually reduced by about three-fourths when the silver was dissolved in 1 M phosphate buffer.

The effect of various salts and pH on the adsorption of Ag^{110m} by Pyrex is shown in Table 9. These results suggest that silver is attracted to ionized groups in the glass surface. The inhibitory effects of the cations are greatest for those having radii similar to those of hydrated silver ions. From the amount of Ag^{110m} adsorbed overnight at 4° C as compared to that at 60° C it is doubtful if there is any temperature-related difference in silver adsorption at pH 6.7-6.8. The effect of temperature on the adsorption rate was not determined.

In a limited number of tests, Vycor flasks adsorbed about 0.5 percent of the Ag^{110m} from 50 ppb solutions at higher pH ranges, while fused silica flasks adsorbed a little less than the Vycor. With continued use, the capacity of the Vycor flasks to adsorb silver progressively increased while under corresponding conditions of use that of the silica flasks increased only slightly. Because only 2 silica flasks were available, they were reused many more times than the Vycor. Therefore, the lesser increase in silver adsorption, with continued use, by fused silica flasks is considered significant.

In parallel tests using Pyrex, Vycor, and quartz flasks in both the original and modified Just and Szniolis (1936) spot test for silver much more silver was recovered in tests with quartz flasks than in those with either Vycor or Pyrex. However, in these tests the silver was added as $AgNO_3$ and precipitated either as AgI or Ag_2S. Therefore, the physical state of the silver was entirely different from that used in the preceding adsorption tests. Consequently, other phenomena, in addition to ionization, may have influenced the loss of silver in these tests.

Based on data obtained in a limited preliminary examination of a single pipette with 1 observation at each time interval, and 3 observations with a second pipette, it appears that the adsorption of Ag^{110m} by borosilicate glass at room temperature is relatively slow (Table 10). This suggests a high energy barrier, which is fairly common for adsorption phenomena. The slow rate of exchange of adsorbed Ag^{110m} with untagged silver, right hand columns of Table 10, also supports the high energy concept. For purposes of comparison it is assumed that essentially all of the Ag^{110m} which can be adsorbed is adsorbed within 18 hours; about 10 percent of this is taken up in the first few seconds, Table 10, while 25 percent is adsorbed in the first 5 minutes.

TABLE 9. THE EFFECT OF pH AND VARIOUS SALTS ON ADSORPTION, FROM A 100 ppb SOLUTION, OF Ag^{110m} BY PYREX GLASS

pH(3)	Average(1) percent of silver adsorbed from solutions of the following salts:(2)										
	Distilled H_2O	$LiNO_3$ M/30	$NaNO_3$ M/30	KNO_3 M/30	NH_4NO_3 M/30	$CaNO_3$ M/30	$Mg(NO_3)_2$ M/30	H_2SO_4	Na_2SO_4 M/15	KNO_3 M/30 - H_3PO_4	PO_4 Buffer M/150 (Na & K)
7.5									0.33		1.85
6.0	11.0*	6.30	0.34	1.70	3.34*				0.53		1.90
5.0	8.45	4.78	0.36		2.48	2.12	6.10*				
4.0	3.30	2.42	0.26	1.35	1.45	1.06	2.94				
3.0	1.48		0.18								
2.0	0.48	0.47*	0.16	0.44*	0.30	0.26	0.40	0.14*		0.13	
1.0	0.13	0.08						0.09	0.11		
0.3	0.02	0.12*	0.04	0.02	<0.01	0.02	<0.01				

(1) The average of results obtained with 2 flasks, results indicated by an asterisk are based on 1 flask, and those marked * on 4 flasks.

(2) Molarities refer to the salts.

(3) The appropriate acid or alkali was used to adjust pH. The pH values recorded are approximate, being ± 0.3-0.4 at the higher pH's. At progressively lower pH levels the ± range gradually narrowed.

TABLE 10. RATE OF UPTAKE OF IONIC SILVER FROM SOLUTION AND EXCHANGE OF ADSORBED RADIOACTIVE SILVER WITH NONLABELED SILVER IN SOLUTION

Uptake: Use labeled silver nitrate solution (Ag^{110m}), draw into pipette, hold for designated time, rinse with distilled water, dry outside of pipette, count with pipette beside GM tube. The counts represent adsorbed Ag^{110m} that is not removed by one quick rinse.

Exchange: Strong, 0.1% or 1.0% unlabeled silver nitrate solution handled as in uptake experiment. Reduction in counts represents adsorbed Ag^{110m} replaced by unlabeled Ag plus the small amount removed by rinsing. Exchange rate indicates how tightly the silver ions are bound by the glass.

Time	Cumulative percentage uptake of Ag^{110m}	Cumulative percentage of exchange (replacement) of Ag^{110m} by untagged Ag	
	Pipette 1(1)	Pipette 1(2)	Pipette 2(3)
1/6 Min.	9	33	...
1/4 Min.	...	...	54
1/2 Min.	10	53	...
3/4 Min.	...	...	60
1-1/2 Min.	...	63	...
3-1/2 Min.	...	...	69
5-1/2 Min.	25	...	...
7 Min.	...	72	...
32 Min.	...	78	...
140 Min.	...	84	...
18 Hours	100	91	...

(1) Percentage uptake with the 2nd pipette was not recorded at the various times.
(2) Untagged exchange solution was 0.1 percent $AgNO_3$.
(3) Untagged exchange solution was 1.0 percent $AgNO_3$.

ADSORPTION ON PYREX GLASS

Results obtained in adsorption studies using Pyrex test tubes exposed to Ag^{110m} solutions in a range from 0.02 ppb to 2000 ppm are presented in Figure 2. This is a plot of the silver adsorbed by the surface of the test tubes against the concentration of silver left in the solution after 18 hours equilibration time. The 25 x 150 mm tubes contained 50 ml of neutral labeled silver nitrate. Tubes stood overnight at 23-26° C, then were rinsed for about 30 seconds with running water, then 3 times with distilled water and dried with acetone. For the simplest type of adsorption, in which the adsorbing sites are identical and independent, the equilibrium constant (K) can be expressed as: $K = \frac{ab_n}{a \cdot b^n}$ a representing total adsorption sites, b the concentration of adsorbate and ab_n sites which have each adsorbed n adsorbate ions or molecules. This equation, in logarithmic form, was used to plot the data presented in Figure 3. With Pyrex glass the apparent order of reaction with respect to silver was 1/2 and the equilibrium constant 3.2×10^2 molar$^{-1/2}$ for adsorption of silver from neutral solution. Adsorption by Pyrex increases with increasing Ag^{110m} concentration, and approaches a maximum value of about 3 μg. of silver per test tube (25 x 150 mm Pyrex in which 90 cm^2 of the surface area is exposed to 50 ml of silver solution) from a neutral solution. About half of the maximum adsorption occurs with a 0.5 ppm solution. The loss of Ag^{110m} from 0.2 ppm (200 ppb) solution

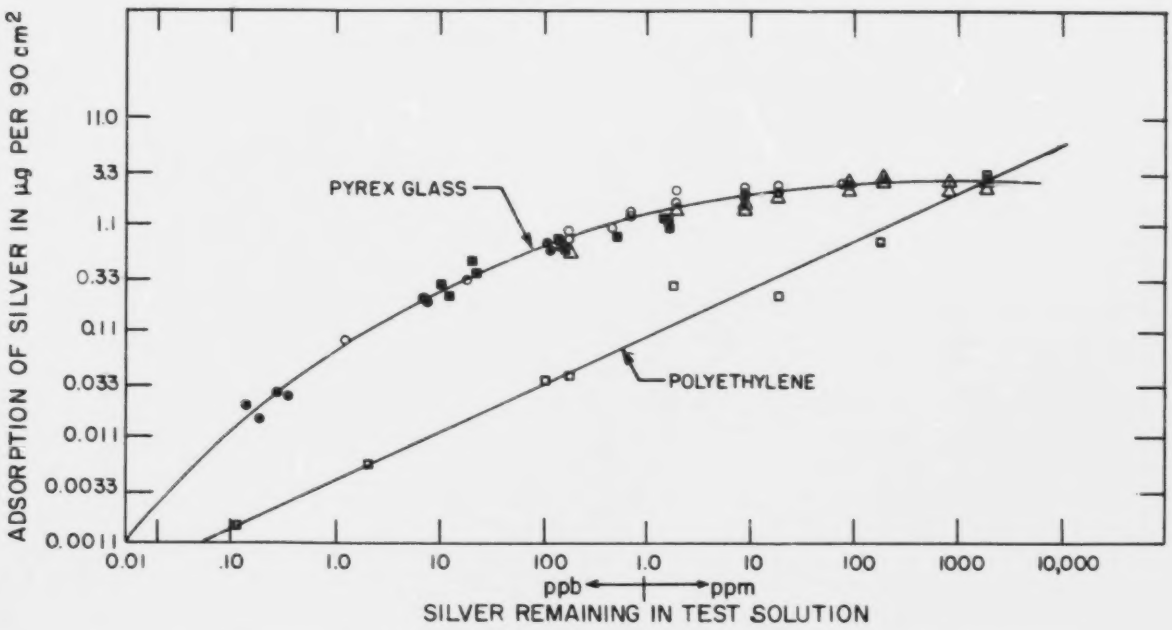

Figure 2. EFFECT OF CONCENTRATION ON ADSORPTION OF SILVER BY PYREX AND HEAT RESISTANT POLYETHYLENE TEST TUBES

noted in Table 7 was equivalent to 5.4 ppb and is not comparable to the losses reported in Figures 2 and 3 and differs greatly from the 15-45 percent (15-45 ppb) losses reported with 100 ppb Ag^{110m} (p. 6). The discrepancy between these results cannot be resolved on the basis of results presently available.

ADSORPTION ON OTHER MATERIALS

A polyethylene graduate which was filled with a Ag^{110m} solution and allowed to stand 75 minutes, followed by 3 rinses each with water and acetone, retained a significant amount of silver. When this graduate was subsequently filled with water and allowed to stand overnight, 30 percent of the adsorbed silver migrated to the water. Heat-resistant polyethylene tubes have lower affinity for silver than Pyrex but showed no tendency toward saturation at concentrations up to 1500 ppm (straight line in Figure 2).

Plastic films (Goodyear Pliofilm and Saran types 5 and 517) and Tygon tubing all adsorbed significant amounts of Ag^{110m}. The apparent order of reaction for the adsorption of silver by the polyethylene tubes was also 1/2. However, these experiments were not carried to significantly high concentrations to provide a value for b. Therefore, a constant could not be calculated.

Membrane filters are used in some procedures for the bacteriological examination of water. One hundred ml samples of Cincinnati tap water and distilled water containing 100 ppb of Ag^{110m} were filtered through type HA Millipore filters. From 1.0 to 2.0 percent of the silver was removed from the distilled water by the membrane. This is equivalent to a silver concentration of from 50 to 100 ppb when the usual 2.0 ml of media is subsequently added to the filter pad. Thirty percent of the silver was removed from the tap water and the same result was obtained when 100 ppb of silver was added to tap water previously filtered through a membrane filter, showing that particulate material was apparently not responsible for the increased removal of silver from the tap water. The addition of a complexing agent (500 ppm of $Na_2S_2O_3 \cdot 5H_2O$) to 100 ppb solutions of silver in tap and distilled water reduced the removal of silver by the membrane filter to 0.2-0.3 per cent from either sample. This is equivalent to 10-15 ppb of silver when 2.0 ml of media is added to the filter pad.

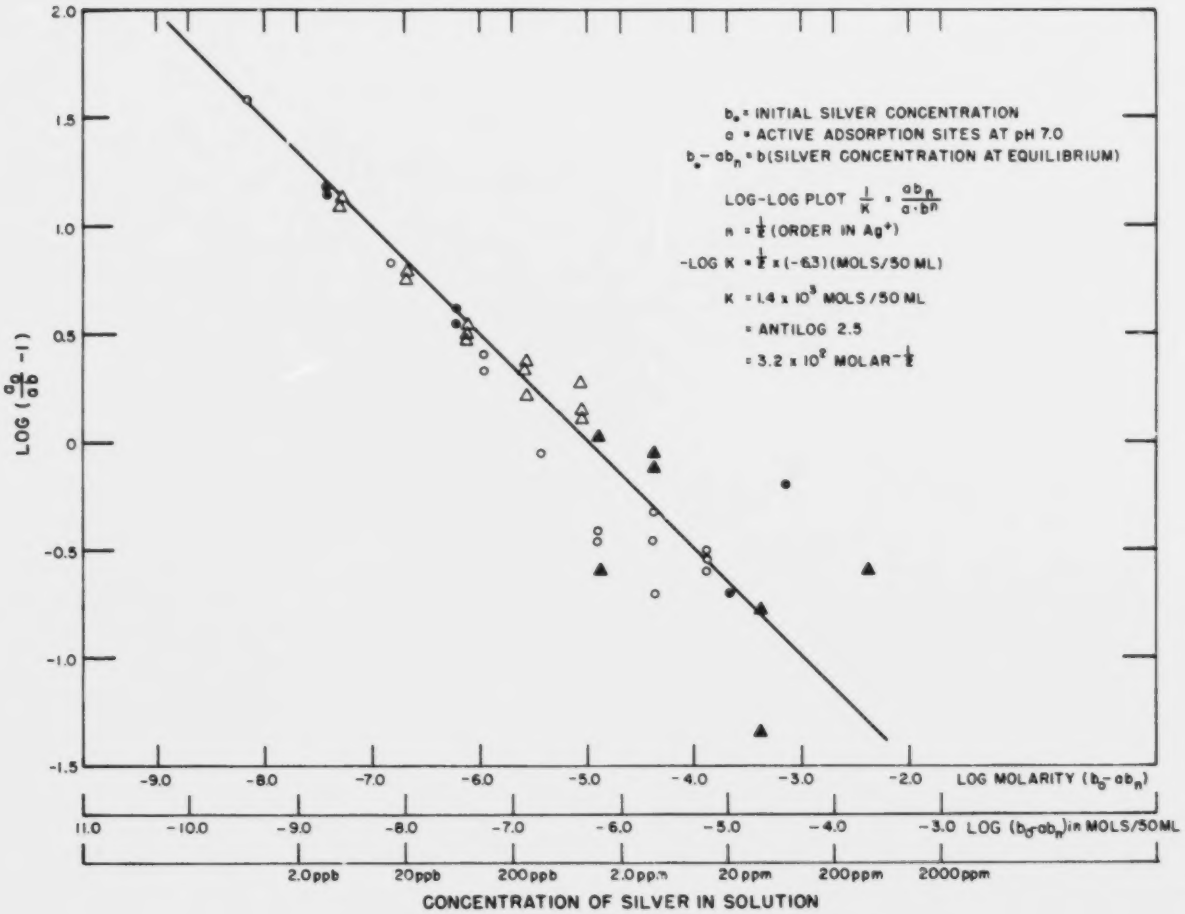
b_o = INITIAL SILVER CONCENTRATION
a = ACTIVE ADSORPTION SITES AT pH 7.0
$b_o - ab_n$ = b(SILVER CONCENTRATION AT EQUILIBRIUM)
LOG-LOG PLOT $\frac{1}{K} = \frac{ab_n}{a \cdot b^n}$
$n = \frac{1}{2}$ (ORDER IN Ag^+)
$-\text{LOG } K = \frac{1}{2} \times (-6.3)$ (MOLS/50 ML)
$K = 1.4 \times 10^3$ MOLS/50 ML
= ANTILOG 2.5
$= 3.2 \times 10^2$ MOLAR$^{-\frac{1}{2}}$
LOG $(\frac{a}{ab} - 1)$
LOG MOLARITY $(b_o - ab_n)$
LOG $(b_o - ab_n)$ in MOLS/50ML
2.0ppb 20ppb 200ppb 2.0ppm 20ppm 200ppm 2000ppm
CONCENTRATION OF SILVER IN SOLUTION

Figure 3. PLOT TO DETERMINE APPARENT REACTION ORDER AND EQUILIBRIUM CONSTANT FOR ADSORPTION BY PYREX SHOWN IN FIGURE 2

Removal of residual silver

Untagged silver was used in much of the work dealing with residual silver toxicity tests with bacteria. Bactericidal evaluations of residual silver were made by adding test water to tubes, allowing time for the test water to leach silver from the glass. Bacterial suspensions were then added and aliquots were removed at increasing time intervals to determine whether residual toxicity was present.

Test tubes contaminated by Ag^{110m} were coated with a heavy slurry of bakers yeast. In related bacteriological tests, tubes were contaminated by 24 hour contact with 1.0 percent Ag as $AgNO_3$ or a 1 to 10 dilution of a colloidal silver preparation, rinsed, filled with a 24-hour culture of baker's yeast in Saboraud's broth. Parallel control tubes filled with uninoculated broth were also prepared. After 72 hours tubes were rinsed and autoclaved 15 minutes at 121°C. Sterile 1-1/2 x B.O.D. dilution water A. P. H. A. (1946) was added and allowed to stand 24 hours. These data are presented in Table 11. Poor Ag^{110m} removal was noted in the radio-assay tests.

TABLE 11.(1) DETOXIFICATION OF PYREX WITH YEAST

Germicide(2)	Detoxifying agent	Percent of bacteria surviving for:		
		2 Hrs.	4 Hrs.	6 Hrs.
None	None	100.0	100.0	95.4
$AgNO_3$	Yeast	97.7	77.9	68.2
$AgNO_3$	Broth control	100.0	100.0	88.0
Colloidal(3)	Yeast	49.0	40.6	38.4
Colloidal	Broth control	87.6	79.0	69.7

(1) Representative experiment.
(2) Tubes contained either 10 ml of 1.0 percent $AgNO_3$ or 15 ml of colloidal material during contamination.
(3) 1:10 dilution of commercial stock.

The efficiency of dichromate cleaning solution for removing Ag^{110m} was determined by parallel radiological assay and bacterial toxicity methods. Data obtained in these studies are presented in Table 7 where it is shown that dichromate solution is inefficient in removing the bactericidal silver (see page 5).

Because Chambers, Chambers, and Kabler (1953) reported that NaCl was a good silver desorbing agent, solutions of NaCl were reinvestigated under a wider variety of conditions in tubes contaminated with Ag^{110m} in concentrations ranging from less than 1 ppm to a maximum of 0.2 percent. The effect of boiling time on the removal of silver by NaCl is shown in Table 12. Even though good removal was obtained in 1 hour, equipment was boiled 2-3 hours in routine practice and allowed to cool overnight to provide a safety factor.

When NaCl concentrations of 0.01, 0.05, 0.25, 1.25 percent, as well as a saturated solution, were tested in parallel at pH 7.7-8.2 differences in efficiency due to varying the NaCl concentration were not noted. However, because of certain limitations of the tests with lower salt concentrations, it is considered desirable to use a 5-10 percent solution. Although a possible slight improvement in removal appeared to result when the pH was increased to 8.0-11.0, lower ranges of pH (2.0-5.0) were adequate at the times recommended (2-3 hours) and prevented precipitation of impurities present in the commercial grades of salt used.

TABLE 12. EFFECT OF BOILING TIME ON THE REMOVAL OF SILVER BY SATURATED NaCl at pH 7.2-7.7

Boiling time	Average percent of silver removed from tubes contaminated with:	
	10 ppb Ag^{110m}	35 ppb Ag^{110m}
5 Min.	93	85
15 Min.	94	88
30 Min.	99	98
60 Min.	100	>99
120 Min.	100	>99

Saran (types 5 and 517) and Tygon were effectively cleaned by a 2 day soak in acid NaCl. Silver was not completely removed from Pliofilm similarly treated.

Tubes contaminated with Ag^{110m} were exposed simultaneously to concentrated ultraviolet light and daylight for 4 days. Results obtained in subsequent silver removal tests indicated that the degree of removal was unaffected by the kind of light exposure.

A comparison of the bactericidal effect of silver leached from tubes, cleaned by several methods is presented in Table 13. Tubes containing high concentrations of silver were used in this work in order to develop an adequate method for detoxifying glassware contaminated with stock solutions. The only completely effective method of removing all types of silver studied is boiling in NaCl solution. The effect of increased leaching time beyond 24 hours is inconclusive, although there appears to be a slight trend toward progressive increase in desorption of silver during the 7 day interval.

TABLE 13. EFFECT OF EXTENDED LEACHING TIME ON TOXICITY OF TUBES CLEANED BY DIFFERENT METHODS

Expt. No.	Leaching time in days	Toxifying(1) material	Percent of bacteria surviving for time indicated											
			2 Hours				4 Hours				6 Hours			
			Water rinse	Hot(2) NaCl	Cold(3) NaCl	Cold(4) Na_2SO_3	Water rinse	Hot(2) NaCl	Cold(3) NaCl	Cold(4) Na_2SO_3	Water rinse	Hot(2) NaCl	Cold(3) NaCl	Cold(4) Na_2SO_3
13	1	None	98.0				93.9				92.9			
		$AgNO_3$	69.6	100.0	98.3	77.6	56.8	92.2	98.3	46.6	59.8	94.1	100.0	35.6
		Colloidal	33.0	100.0	45.6	75.1	11.8	100.0	29.2	36.1	17.7	97.6	22.2	28.7
14	2	None	100.0				100.0				100.0			
		$AgNO_3$	47.0	96.7	93.3	62.1	27.1	86.4	88.6	26.4	16.0	82.2	86.0	18.0
		Colloidal	19.0	100.0	44.2	49.0	12.7	98.8	16.7	27.6	10.8	94.5	16.7	21.4
15	3	None	100.0				100.0				93.2			
		$AgNO_3$	69.8	95.4	100.0	91.6	65.8	90.6	89.6	61.8	54.6	94.4	86.0	50.7
		Colloidal	31.6	93.9	68.8	76.7	21.0	84.4	28.8	50.8	16.2	84.0	25.4	42.8
16	4	None	94.8				95.2				86.0			
		$AgNO_3$	83.2	96.4	94.4	73.2	63.4	91.5	89.7	49.6	54.2	95.3	83.3	42.4
		Colloidal	...	90.8	29.2	25.3	...	94.4	16.7	19.2	...	86.6	13.4	15.0
18	6	None	94.3				99.5				91.6			
		$AgNO_3$	27.2	96.5	94.5	57.7	12.7	77.4	93.8	33.7	7.4	83.1	86.0	26.0
		Colloidal	9.79	94.6	24.6	18.0	5.58	89.2	11.2	8.84	1.40	86.0	9.25	5.55
19	7	None	81.6				87.5				86.2			
		$AgNO_3$	13.6	82.2	96.6	38.4	7.60	71.5	91.2	15.6	3.95	74.8	75.2	11.7
		Colloidal	8.68	18.6	18.6	9.36	4.32	87.4	7.75	5.85	6.20	86.7	7.15	4.09

(1) Contaminated by 24 hr. contact with 1.00% Ag as $AgNO_3$ or a 1:10 dilution of colloidal concentrate.
(2) Placed in boiling NaCl (saturated + 3.0 ml conc. HCl and 0.1 g $CuSO_4$ per liter), boil 20 min., cool overnight and allow total contact of 24 hr., rinse in tap water and distilled water.
(3) Same as (2) except without boiling (elapsed time 24 hr.).
(4) Soak for 24 hrs. in 5% Na_2SO_3 solution then rinse with tap water and distilled water.

Summary

Results of this investigation can be summarized as follows:

1. In carefully conducted tests, it appears that discrepant bactericidal results occur more frequently with silver than with other germicides.

2. In the experiments conducted the germicidal action of a specified amount of silver was found to be related to the concentration of silver ions rather than to the physical nature of the silver from which the ions are originally derived.

3. The presence or absence of light or the density of test organisms within the range of 1,700 to 1,700,000/ml produces no discernible effect on the bactericidal action of silver. Phosphate tends to decrease germicidal efficiency while increases of pH are accompanied by accelerated death rates.

4. When silver contaminated glass is reused, some of the silver is desorbed and exerts a germicidal action.

5. Of several neutralizers studied, a mixture of sodium thiosulfate and sodium thioglycollate was found to be most efficient.

6. In the presence of inefficient neutralizers, bacteriostasis may be mistaken for bactericidal action.

7. The adsorption of silver on surfaces varies not only with the strength and composition of the solution and the kind of surface material, but also with the previous use and treatment of the surface.

8. Hydrogen ions and various other ions such as halides, phosphates, and ammonia reduce the amount of adsorption.

9. Adsorption by Pyrex glass increases with increasing silver concentration up to about 100 ppm and approaches a maximum value after 18 to 24 hours.

10. Boiling in sodium chloride solution is the most effective method of removing the adsorbed silver.

11. Radioactive Ag^{110m} is useful in determining silver concentration in solution and the residuals remaining on surfaces.

12. Chromatographic methods for silver separation prior to analysis did not prove satisfactory under the conditions of this study (See Appendix B).

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Appendix A. Radioassay method

The Ag^{110m} used in this study was received in nitric acid solution with a specific activity of 535 mc/g. In all instances where Ag^{110m} is referred to, it was used as $AgNO_3$, a neutral working stock solution which contained 0.2 mg (or 0.1 mc) per ml serving as the source of the Ag^{110m}. This solution was 200 ppm in silver. When silver concentrations greater than about 1 ppm were needed, the Ag^{110m} was used to tag ordinary silver nitrate solution.

In all tests using Ag^{110m} preliminary studies were made to resolve problems of standardization, shielding, and geometry. In tests where 25 x 150 mm culture tubes were used the number of counts per minute were determined with a G.M. tube (Fig. 4) centered in the culture tube. In tests in which 250 ml Erlenmeyer flasks were used a gamma scintillation counter was used (Fig. 5). Gamma photons from Ag^{110m} are only slightly adsorbed by glass and water, so the detector could be outside of the flask. Tests indicated that the same counting efficiency was found for a dry flask that had absorbed Ag^{110m} from 100 ml of solution and for the same flask with solution after the Ag^{110m} had been desorbed.

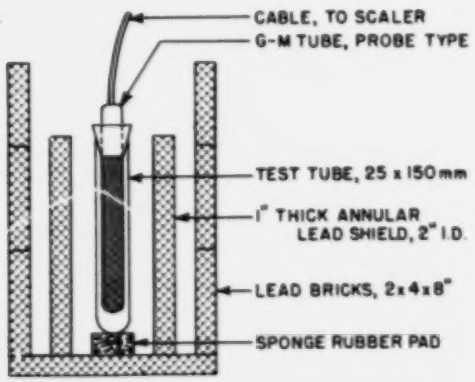
CABLE, TO SCALER
G-M TUBE, PROBE TYPE
TEST TUBE, 25 x 150 mm
1" THICK ANNULAR LEAD SHIELD, 2" I.D.
LEAD BRICKS, 2x4x8"
SPONGE RUBBER PAD

Figure 4. SET-UP FOR STUDIES OF ADSORPTION BY GLASSWARE AND OF CLEANING METHODS. Except for depth, position of the G-M probe in the test tube did not affect counting efficiency.

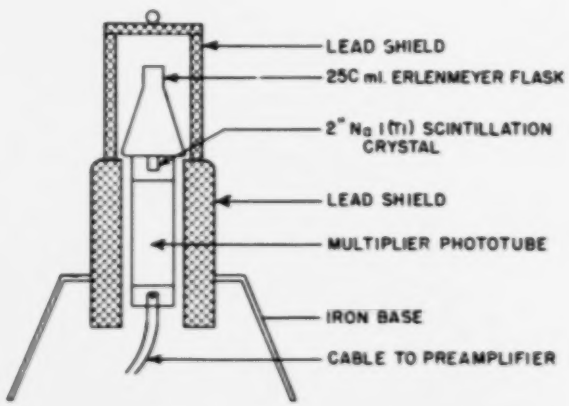

Figure 5. ARRANGEMENT FOR γ-SCINTILLATION COUNTING OF Ag^{110m} IN FLASKS. A Radiation Counter Laboratories single channel analyzer was used.

Appendix B. Chemical methods

The rhodanine method described by Feigl (1928) was tried in its original form and in a variety of modifications using both p-dimethyl and p-diethylaminobenzalrhodanine as a color reagent. Difficulty with precipitation of the reagent was encountered with the dimethyl form while the diethyl compound was significantly more sensitive and precipitation was not as serious a problem. The best results were obtained with the following modification of this test: Transfer 10.0 ml of a buffer solution consisting of 20 percent (w/v) Na_2SO_4 in 1.0 percent (v/v) H_2SO_4, to a 100 ml Nessler tube. Add sample to the 100 ml mark and mix well by inverting 4 times, using a Pliofilm closure. For concentrations of 20-70 ppb of silver add 0.6 ml of a 0.05 percent alcoholic (absolute ethyl) solution of p-diethylaminobenzalrhodanine beneath the surface and immediately mix as above. Allow to stand for 20 minutes or more and compare with a series of standards prepared at the same time and in exactly the same manner. The volume of color reagent to be added bears a critical relationship to the concentration of silver in the sample. Accordingly, suitable volumes of reagent for other silver concentration ranges should be determined by preliminary tests.

In preparing standards for the rhodanine test it is desirable to provide a series of uniformly increasing silver concentrations covering 5 or more concentrations. The tubes used should first be examined by running a blank test, using all reagents, to determine that there is no residual silver in the tubes; even with these control procedures "sleepers" frequently occur. However, in a series of standards with uniformly increasing silver concentrations, misfits can readily be spotted and a new set of standards prepared. When this method was used to control silver concentrations in bactericidal tests distilled water was used in order to avoid interferences from mineral content. Interference was noted in a limited number of tests with Cincinnati tap water. The bacteria used in the bactericidal examinations did not affect the test.

The method of Just and Szniolis (1936), as modified by Chesney and Renn (1955), was examined. In this method 100 ml of sample is acidulated with 10 drops of 1:1 HCl (v/v) followed by addition of 2-3 drops of 10 percent (w/v) KI. The AgI precipitated is collected on an HA Millipore filter, using a lucite shield to restrict the filter area to a 1.0 cm^2 circle. Three drops of a freshly prepared 500 ppm $Na_2S \cdot H_2O$ solution is added to the spot to convert the AgI to Ag_2S. The resulting spot is then compared with previously prepared standards. The amount of silver recovered by this test could be substantially increased by shaking at 275 oscillations per minute for 5 minutes after adding the HCl and KI. This was the minimum shaking time that was adequate and no marked improvement was noted when it was increased to 15-20 minutes.

In the present study this test was further modified for use in distilled water by adding all reagents before filtration. A better silver yield was obtained under these conditions and this was further increased by shaking. However, this modification cannot be used in naturally occurring waters because of the danger of interference from such metals as lead and copper. A much higher silver yield was obtained with fused quartz flasks than with Vycor or Pyrex when compared in parallel tests because Vycor and Pyrex adsorbed more of the silver. Use of a PH (finer) Millipore filter further increased the silver yield while the still less porous VC filter was too slow to be practical. However, in tests with Ag^{110m} in distilled water, with all reagents added before shaking, approximately 20 percent of the Ag^{110m} remained in the filtrate even when the PH filter was used.

Column chromatography was investigated using dithizone-treated cellulose acetate (usually 25-35 mesh) in a 1.0 cm diameter column (Carritt 1953). A variety of column lengths and experimental conditions were evaluated for their efficiency in adsorption and recovery of

Ag^{110m}. Over 20 different eluting agents were tried, none of which were very satisfactory. The best adsorption (99.4 percent) was obtained with a column containing 1.0 cm. of treated cellulose acetate over 1/2 cm of the untreated material. The best recovery (95.4 percent) of Ag^{110m} was also realized with this column by eluting with boiling hot 10 percent NaC1 contained 1000 ppm of chlorine at pH 2.0, followed by boiling 10 percent NaC1 in 1N-HC1. This treatment, as was expected, destroyed the column. A column packed with Pyrex wool adsorbed 97.5 percent of the Ag^{110m} from an alkaline (pH 9.0) solution and 87.3 percent of this Ag^{110m} was recovered by eluting with boiling 10 percent NaC1 in 1N-HC1. The glass wool was removed, placed in a flask and made up to volume to standardize geometric relationships. There was no Ag^{110m} in the glass wool which left 10 percent of the adsorbed silver unaccounted for.

Appendix C. Bacteriological procedures

NEUTRALIZER EXAMINATIONS

The solutions evaluated for neutralizing efficiency were prepared in 15 x 150 mm screw-cap Pyrex tubes. The volume used was such that 9.0 ml of solution remained after steam sterilization for 15 minutes at 121° C. Because Na_2SO_3 is unstable with respect to both oxygen and high temperatures it was necessary to sterilize water for these solutions separately by boiling. The water was quickly cooled, following which Na_2SO_3 was immediately added. Aliquots of the solution were quickly transferred to sterile tubes. This entire procedure was timed so that the experiment could start as soon as the solutions were tubed. Incubated controls established the sterility of Na_2SO_3 solutions so prepared.

In the neutralizer test, 1.0 ml of a stock silver solution as $AgNO_3$ was added to a tube of neutralizer and quickly mixed. One ml of bacterial suspension containing approximately 1500 Escherichia coli ATCC 11229 organisms per ml was then immediately added. Duplicate 1.0 ml agar plate inoculations were made as quickly as possible (within 1 minute). Three replicate tubes at each silver concentration were usually prepared in each experiment. The tubes were stored at 20° C, subsequent inoculations being made from each tube after intervals of 2, 4 and 6 hours respectively. In experiments where duplicate inoculations were made in each of two types of agar at the various time intervals, the concentration of the suspension was doubled and the volume of inoculum reduced to 0.5 ml to provide sufficient material for the increased number of plates. The efficiency of neutralization was based on the degree of protection provided throughout the 6 hour period. Parallel controls established that the unsilvered neutralizer was not toxic.

BACTERICIDAL EFFICIENCY TESTS

In the germicidal test (Chambers 1956), 99.0 ml of test water containing the desired concentration of silver was added to each of a series of 250 ml wide mouth Pyrex Erlenmeyer flasks. One ml of a suspension containing approximately 2,000,000 E. coli ATCC 11229 organisms per ml was added to each flask, including control flasks without germicide. After the desired exposure intervals 1.0 ml aliquots were transferred to 9.0 ml of neutralizer and mixed. Duplicate agar pour plate inoculations of appropriate volumes were then prepared.

When Ag^{110m} was used in bactericidal tests, counts were made on each flask before adding the Ag^{110m}. Immediately after adding the silver solution another reading was taken. Solutions were then stabilized at 20° C, bacterial suspension being added immediately thereafter. When the last aliquot was withdrawn for bacteriological examination the flask was emptied, quickly rinsed with water, and dried with an acetone rinse. By comparing the initial and final readings, the amount of Ag^{110m} adsorbed by the glass, as well as that remaining in solution, could be calculated. However, because silver was gradually being adsorbed by the glass throughout the experiment, the concentration of silver in solution at the time of the final reading was less than at any time during the experiment. In order to minimize changes resulting from the adsorption of silver during the bacteriological tests, Ag^{110m} solutions were allowed to stand overnight (sometimes longer) in the test flasks in order for the solution to more nearly reach equilibrium before test organisms were added.